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(54) Title: INHIBITION OF *CANDIDA*

## (57) Abstract

Compositions and methods are provided for the treatment and diagnosis of *Candida* infections. In accordance with preferred embodiments, oligonucleotides and oligonucleotide analogs are provided which are specifically hybridizable with at least a portion of a *Candida* mRNA. Preferred targets are the mRNAs which encode  $\beta$ -tubulin, aspartate protease, actin and chitin synthetase, as well as the mRNA's which encode the ribosomal L25 protein, translation elongation factors 1 and 2 (TEF1 and TEF2), the b subunit of ATPase, and cytochrome P450 lanosterol 14 $\alpha$ -demethylase (L1A1). The oligonucleotides and oligonucleotide analogs comprise nucleotide units sufficient in identity and number to effect said specific hybridization. In other preferred embodiments, the oligonucleotides are specifically hybridizable with a transcription initiation site, a translation initiation site, 5'-untranslated sequences, 3'-untranslated sequences, 5'-cap, and intron/exon junction of the mRNAs. Methods of treating animals suffering from *Candida* infection are disclosed.

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<sup>+</sup> Any designation of "SU" has effect in the Russian Federation. It is not yet known whether any such designation has effect in other States of the former Soviet Union.

## INHIBITION OF CANDIDA

### FIELD OF THE INVENTION

This invention relates to diagnostics, research reagents, and therapies for *Candida* infections. In particular, this invention relates to antisense oligonucleotide interactions with certain *Candida* messenger ribonucleic acids. Specifically, antisense oligonucleotides are designed to hybridize to the *Candida* mRNA's which encode the  $\beta$ -tubulin, actin, chitin synthetase and aspartate protease proteins. Other antisense oligonucleotides are designed to hybridize specifically to the *Candida* mRNA's which encode the ribosomal L25 protein, translation elongation factors 1 and 2 (TEF1 and TEF2), the b subunit of ATPase, and cytochrome P450 lanosterol 14 $\alpha$ -demethylase (L1A1). These oligonucleotides have been found to lead to the modulation of the activity of the *Candida* RNA or DNA, and thus to the modulation of the *Candida* infection. Palliation and therapeutic effect result.

### BACKGROUND OF THE INVENTION

Opportunistic infections in immunocompromised hosts represent an increasingly important cause of mortality and morbidity. *Candida* species are among the most common of the fungal pathogens with *Candida albicans* as the most common species, but with *Candida tropicalis*, *Candida krusei*, *Candida glabrata* (*Torulopsis glabrata*) and *Candida parapsilosis* also found in infected individuals. *Candida* is responsible for a variety of nosocomial infections. For a general review of the types & severity

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of *Candida* infections see Meunier, F., *Eur. J. Clin. Microbiol. Infect. Dis.* 8:438-447 (A89) or Radentz, W., *J. Am. Acad. Derm.* 20:989-1003. Cancer patients, particularly leukemia patients, are at high risk of *Candida* infections.

- 5 Up to 30% of all leukemia patients show evidence of invasive candidiasis upon autopsy. Cancer patients with a variety of solid tumors also are at risk of opportunistic infection. Improvements in the treatment of cancer, with greater use of surgery and newer chemotherapies, has  
10 resulted in increasing numbers of non-terminal patients becoming infected with *Candida* and requiring treatment.

- Another group at risk for *Candida* and other opportunistic infections is the AIDS population. In AIDS patients *Candida* is a problem in oropharyngeal infections.  
15 Burn patients, I.V. drug users, persons with catheters and premature neonates are all also susceptible to infection by *Candida*.

- Candida* can also be problematic in the non-immunocompromised host. In normal healthy women, *Candida*  
20 is responsible for vulvovaginitis. The overwhelming majority of yeasts which infect the vagina are isolates of *Candida albicans*. This problem is often exacerbated by pregnancy, the use of oral contraceptives or in disease situations requiring the use of antibiotics, all of which  
25 increase the probability of an infection by *Candida*.

- There are currently several drugs in use for managing *Candida* infections. Amphotericin B is generally considered the standard therapy for systemic *Candida* infection. However, amphotericin B has a number of severe  
30 side effects, some of which cause permanent damage to the patients' liver and kidneys. Moreover, the efficacy of amphotericin B is limited and treatment does not always result in elimination of the infection. Therefore, there is a great need for agents which are effective in  
35 inhibiting *Candida* infections but do not cause toxic side effects to the host. Antisense oligonucleotides hold great promise as therapeutic agents for *Candida* infections.

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There have been no prior attempts to inhibit *Candida* with antisense oligonucleotides. Accordingly, there has been and continues to be a long-felt need for the design of oligonucleotide analogs which are capable of effective therapeutic use.

#### OBJECTS OF THE INVENTION

It is an object of this invention to provide oligonucleotides and oligonucleotide analogs which are capable of hybridizing with messenger RNA of *Candida* to inhibit the function of the messenger RNA.

It is a further object to provide oligonucleotides and analogs which can modulate the expression of *Candida* through antisense interaction with messenger RNA of the fungus.

Yet another object of this invention is to provide methods of diagnostics and therapeutics for *Candida* in animals. Methods, materials and kits for detecting the presence or absence of *Candida* in a sample suspected of containing it are further objects of the invention.

Novel oligonucleotides and oligonucleotide analogs are other objects of the invention.

These and other objects will become apparent to persons of ordinary skill in the art from a review of the instant specification and appended claims.

#### SUMMARY OF THE INVENTION

In accordance with the present invention, oligonucleotides and oligonucleotide analogs are provided which specifically hybridize with at least a portion of an RNA from *Candida*. The oligonucleotide or oligonucleotide analog is preferably designed to bind directly to *Candida* RNA.

This relationship is commonly denoted as "antisense." The oligonucleotides and oligonucleotide analogs are able to inhibit the function of RNA -- either its translation into protein, its translocation into the cytoplasm, or any other activity necessary to its overall biological function. The failure of the RNA to perform all

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or part of its function results in failure of a portion of the genome controlling the normal life cycle of the fungus.

It has been found to be preferred to target specific *Candida* RNA portions for antisense oligonucleotide attack. It has been discovered that the genes coding for  $\beta$ -tubulin, aspartate protease, actin and chitin synthetase are particularly useful for this approach. The genes encoding the ribosomal L25 protein, translation elongation factors 1 and 2 (TEF1 and TEF2), the b subunit of ATPase, and cytochrome P450 lanosterol 14 $\alpha$ -demethylase (L1A1) are also particularly useful. Inhibiting translation of the mRNA's relating to these proteins is expected to be useful for the treatment of *Candida* infections.

Methods of modulating *Candida* infection comprising contacting the animal with an oligonucleotide or oligonucleotide analog hybridizable with nucleic acid of the fungus are provided. Oligonucleotides or analogs hybridizable with mRNA coding for  $\beta$ -tubulin, aspartate protease, actin and chitin synthetase proteins are preferred. Oligonucleotides or analogs hybridizable with mRNA coding for the ribosomal L25 protein, TEF1 and TEF2, the ATPase b subunit and cytochrome P450 L1A1 are also preferred.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is the sequence of the  $\beta$ -tubulin gene of *Candida albicans*.

Figures 2 A and B are graphical representations of the effects of antisense oligonucleotides on *Candida* germ tube formation at oligonucleotide doses of 0.5 (A) and 1.0  $\mu$ M (B).

#### DETAILED DESCRIPTION OF THE INVENTION

Antisense oligonucleotides hold great promise as therapeutic agents for the treatment of many human diseases. Oligonucleotides specifically bind to the complementary sequence of either pre-mRNA or mature mRNA, as defined by Watson-Crick base pairing, inhibiting the flow of genetic information from DNA to protein. Numerous

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recent studies have documented the utility of antisense oligonucleotides as biochemical tools for studying target proteins. Rothenberg et al., *J. Natl. Cancer Inst.*, 81:1539-1544 (1989); Zon, G., *Pharmaceutical Res.* 5:539-549 (1988). Because of recent advances in oligonucleotide chemistry, synthesis of nuclease resistant oligonucleotides, and availability of types of oligonucleotide analogs which exhibit enhanced cell uptake, it is now possible to consider the use of antisense oligonucleotides as a novel form of therapeutics.

For therapeutics, an animal suspected of having a *Candida* infection is treated by administering oligonucleotides or oligonucleotide analogs in accordance with this invention. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. Such treatment is generally continued until either a cure is effected or a diminution in the disease state is achieved.

It is to be expected that differences in the DNA of *Candida* from different species and from different types within a species exist. Thus, it is believed, for example, that the regions of the various *Candida* species serve essentially the same function for the respective species and that interference with expression of the genetic information will afford similar results in the various species. This is believed to be so even though differences in the nucleotide sequences among the species doubtless exist.

Accordingly, nucleotide sequences set forth in the present specification will be understood to be representational for the particular species being described. Homologous or analogous sequences for different species of *Candida* are specifically contemplated as being within the scope of this invention.

The present invention employs oligonucleotides and oligonucleotide analogs for use in antisense inhibition of the function of *Candida* RNA. In the context of this

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invention, the term "oligonucleotide" refers to a polynucleotide formed from naturally occurring bases and pentafuranosyl groups joined by native phosphodiester bonds. This term effectively refers to naturally occurring  
5 species or synthetic species formed from naturally occurring subunits or their close homologs.

"Oligonucleotide analog", as that term is used in connection with this invention, refers to moieties which function similarly to oligonucleotides but which have non-  
10 naturally occurring portions. Thus, oligonucleotide analogs may have altered sugar moieties or inter-sugar linkages. Exemplary among these are the phosphorothioate and other sulfur-containing species which are known for use in the art. In accordance with some preferred embodiments,  
15 at least some of the phosphodiester bonds of the oligonucleotide have been substituted with a structure which functions to enhance the ability of the compositions to penetrate into the region of cells where the RNA or DNA whose activity to be modulated is located. It is preferred  
20 that such substitutions comprise phosphorothioate bonds, methyl phosphonate bonds, or short chain alkyl or cycloalkyl structures. In accordance with other preferred embodiments, the phosphodiester bonds are substituted with other structures which are, at once, substantially non-  
25 ionic and non-chiral, or with structures which are chiral and enantiomerically specific. Persons of ordinary skill in the art will be able to select other linkages for use in practice of the invention.

Oligonucleotide analogs may also include species  
30 which include at least some modified base forms. Thus, purines and pyrimidines other than those normally found in nature may be so employed. Similarly, modifications on the pentafuranosyl portions of the nucleotide subunits may also occur as long as the essential tenets of this invention are  
35 adhered to.

Such analogs are best described as being functionally interchangeable with natural oligonucleotides



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(or synthesized oligonucleotides along natural lines), but which have one or more differences from natural structure. All such analogs are comprehended by this invention so long as they function effectively to hybridize with *Candida* RNA.

5 The oligonucleotides and oligonucleotide analogs in accordance with this invention preferably comprise from about 3 to about 50 nucleic acid base units. It is more preferred that such oligonucleotides and analogs comprise from about 8 to 25 nucleic acid base units, and still more  
10 preferred to have from about 12 to 25 units. As will be appreciated, a subunit or a nucleic acid base unit is a base-sugar combination suitably bound to adjacent subunits through phosphodiester or other bonds.

The oligonucleotides and analogs used in  
15 accordance with this invention may be conveniently and routinely made through the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including Applied Biosystems. Any other means for such synthesis may also be employed; however, the  
20 actual synthesis of the oligonucleotides is well within the talents of the routineer. It is also well known to use similar techniques to prepare other oligonucleotide analogs such as the phosphorothioates and alkylated derivatives.

In accordance with this invention, persons of  
25 ordinary skill in the art will understand that messenger RNA includes not only the information to encode a protein using the three letter genetic code, but also associated ribonucleotides which form a region known to such persons as the 5'-untranslated region, the 3'-untranslated region,  
30 the 5' cap and intron/exon junction ribonucleotides. Thus, oligonucleotides and oligonucleotide analogs may be formulated in accordance with this invention which are targeted wholly or in part to these associated ribonucleotides as well as to the informational  
35 ribonucleotides. In preferred embodiments, the oligonucleotide or analog is specifically hybridizable with a transcription initiation site, a translation initiation

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site, an intron/exon junction or sequences in the 3'-untranslated region.

In accordance with this invention, the oligonucleotide is specifically hybridizable with at least  
5 a portion of a nucleic acid of *Candida*. In preferred embodiments, the nucleic acid portion includes the mRNA's which encode  $\beta$ -tubulin, actin, chitin synthetase and aspartate protease proteins. In other preferred  
10 embodiments, the nucleic acid portion includes the mRNA's which encode the ribosomal L25 protein, TEF1 and TEF2, the b subunit of ATPase, and cytochrome P450 L1A1. Oligonucleotides or analogs comprising the corresponding sequence, or part thereof, are useful in the invention. Thus, the oligonucleotides and oligonucleotide analogs of  
15 this invention are designed to be hybridizable with messenger RNA of *Candida*. Such hybridization, when accomplished, interferes with the normal function of the messenger RNA to cause a loss of its utility to the fungus. The functions of messenger RNA to be interfered with  
20 include all vital functions such as translocation of the RNA to the situs for protein translation, actual translation of protein from the RNA, splicing or other processing of the RNA, and possibly even independent catalytic activity which may be engaged in by the RNA. The  
25 overall effect of such interference with the RNA function is to cause the *Candida* to lose the benefit of the RNA and, overall, to experience interference with expression of its genome. Such interference is generally fatal to the fungus.

30 Figure 1 is the sequence of the  $\beta$ -tubulin gene of *Candida albicans*. The sequence for the *Candida albicans*  $\beta$ -tubulin gene is known. Smith et al., *Gene*, 63:53-63 (1988). The gene sequence of *Candida albicans* is known. Au-Young et al., *Molecular Microbiology*, 4:197-207 (1990).  
35 The sequence for the *Candida albicans* actin gene is known as well. Losberger et al., *Nucl. Acid. Res.* 17:9488 (1989). The sequence for the *Candida albicans* aspartyl

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proteinase gene is set forth in Lott et al., *Nucl. Acid Res.*, 17:1779 (1989). The sequence for the *Candida albicans* cytochrome P450 L1A1 is disclosed in Lai et al., *Nucl. Acid. Res.*, 17:804 (1989). The sequences for the  
5 *Candida albicans* elongation factors TEF1 and TEF2 are disclosed in Sundstrom et al., *J. Bacteriol.*, 172:2036 (1990). The sequence of the ribosomal L25 gene is known in *Candida glabrata* (*Torulopsis glabrata*) and *Candida utilis*. Wong et al., *Nucl. Acids Res.*, 18: 1888 (1990); Woudt et  
10 al., *Curr. Genet.*, 12:193 (1987). The gene sequence for the *Candida tropicalis* vacuolar ATPase subunit b is disclosed in Gu et al., *Nucl. Acids Res.*, 18:7446 (1990).

Oligonucleotides or analogs useful in the invention are complementary to and comprise one of these  
15 sequences, or part thereof. Thus, it is preferred to employ any of these oligonucleotides (or their analogs) as set forth above or any of the similar nucleotides which persons of ordinary skill in the art can prepare from knowledge of the preferred antisense targets for the  
20 modulation of the fungal infection.

The oligonucleotides and oligonucleotide analogs of this invention can be used in diagnostics, therapeutics and as research reagents and kits. For therapeutic use, the oligonucleotide or oligonucleotide analog is  
25 administered to an animal suffering from a *Candida* infection. It is generally preferred to apply the therapeutic agent in accordance with this invention topically or intralesionally. Other forms of administration, such as transdermally, or intramuscularly  
30 may also be useful. Inclusion in suppositories is presently believed to be likely to be highly useful. Use of the oligonucleotides and oligonucleotide analogs of this invention in prophylaxis is also likely to be useful. Such may be accomplished, for example, by providing the  
35 medicament as a coating in condoms and the like. Use of pharmacologically acceptable carriers is also preferred for some embodiments.

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The present invention is also useful in diagnostics and in research. Since the oligonucleotides and oligonucleotide analogs of this invention hybridize to nucleic acid from *Candida*, sandwich and other assays can easily be constructed to exploit this fact. Provision of means for detecting hybridization of oligonucleotide or analog with *Candida* present in a sample suspected of containing it can routinely be accomplished. Such provision may include enzyme conjugation, radiolabelling or any other suitable detection systems. Kits for detecting the presence or absence of *Candida* may also be prepared.

Several preferred embodiments of this invention are exemplified in accordance with the following examples. The target mRNA species for modulation relates to the  $\beta$ -tubulin, actin, chitin synthetase and aspartate protease proteins of *Candida*. Other preferred mRNA targets relate to the ribosomal L25 protein, translation elongation factors 1 and 2 (TEF1 and TEF2), the b subunit of ATPase, and cytochrome P450 lanosterol 14 $\alpha$ -demethylase (L1A1). Persons of ordinary skill in the art will appreciate that the present invention is not so limited, however, and that it is generally applicable. The inhibition of these *Candida* RNAs are expected to have significant therapeutic benefits in the treatment of disease. In order to assess the effectiveness of the compositions, an assay or series of assays is required.

The following are intended as nonlimiting examples of some embodiments of the invention.

#### EXAMPLES

##### 30 EXAMPLE 1

Inhibition of *candida albicans* with antisense oligonucleotide analogs complementary to the mRNAs coding for  $\beta$ -tubulin, actin, chitin synthetase and aspartate protease

35 A series of antisense oligonucleotide sequences were selected which are complementary to the *Candida*  $\beta$ -tubulin, aspartate protease, actin and chitin synthetase mRNA's. These are shown in Tabl 1:

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TABLE 1

*Antisense Oligonucleotides Targeted to Candida Albicans*

COMPOUND	SEQUENCE (5' - 3')	TARGET RNA	
1275	CAA TTT CTC TCA TAG TTC TA	Tubulin	initiation of translation
1276	CGG AAC ATA CAA TTT CTC TC	Tubulin	5' splice junction intron 1
1277	CAA AAG CAG TTA GTA TAT TT	Tubulin	splice branch point intron 1
1278	AAA AAT TGT TAG TAA AAT CA	Tubulin	splice branch point intron 2
1279	CTA AAA AAA AGG GCA AAA GC	Tubulin	3' splice junction intron 1
1280	TTC CCA AAA GGC AGC ACC CT	Tubulin	3' splice junction intron 2
1281	ATG ATA ACT GCA TGA TGT TG	Aspartate protease	initiation of translation
1282	GGA AGG ATT CCC GTG TGC GG	Aspartate protease	position 585
1283	AAC AAT ACC TAA ACC TTG GA	Aspartate protease	transcriptional terminator
1284	ACC ACC GTC CAT TTT GAA TG	Actin	initiation of transcription
1285	TTA AAA CAT ACA CCG TCC A	Actin	5' splice site
1286	CTA TAA AAA TGG GTT GTA AT	Actin	branch and 3' splice site
1287	TGT TGT CGA TAA TAT TAC CA	Chitin synthetase	initiation of translation
1288	GTG TAT GTC ATG TTG GTA AA	Chitin synthetase	2nd in-frame met
1289	TTT AGC TCT AAC ATC ACC AC	Chitin synthetase	termination of translation

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*Candida albicans* is grown in a standard broth, such as Sabouraud dextrose broth (Difco) or yeast nitrogen base with glucose added. *Candida* is grown in 1 ml of solution and the antisense oligonucleotide compound is added at 50  
5  $\mu$ M and one half log dilutions thereof. Triplicate tubes are prepared for each dose. Inhibition of *Candida* growth is expected to occur with an I.C.<sub>50</sub> of 1-10  $\mu$ M oligonucleotide compound.

**EXAMPLE 2**

10 Synthesis and characterization of oligonucleotides and analogs: Unmodified DNA oligonucleotides were synthesized on an automated DNA synthesizer (Applied Biosystems model 380B) using standard phosphoramidite chemistry with oxidation by iodine. 8-cyanoethyl-diisopropyl-  
15 phosphoramidites were purchased from Applied Biosystems (Foster City, CA). For phosphorothioate oligonucleotides, the standard oxidation bottle was replaced by a 0.2 M solution of 3H-1,2-benzodithiole-3-one 1,1-dioxide in acetonitrile for the stepwise thiation of the phosphite  
20 linkages. The thiation cycle wait step was increased to 68 seconds and was followed by the capping step.

After cleavage from the controlled pore glass column (Applied Biosystems) and deblocking in concentrated ammonium hydroxide at 55°C for 18 hours, the  
25 oligonucleotides were purified by precipitation twice out of 0.5 M NaCl with 2.5 volumes ethanol. Analytical gel electrophoresis was accomplished in 20% acrylamide, 8 M urea, 45 mM Tris-borate buffer, pH 7.0. Oligodeoxynucleotides and their phosphorothioate analogs  
30 were judged from electrophoresis to be greater than 80% full length material.

**EXAMPLE 3**

Germ tube assay for antisense oligonucleotide inhibition of  
*Candida*: The development of germ tubes, the initial stage  
35 in formation of hyphae, is believed to be important in allowing *Candida* to escape the effect of macrophages. Drugs that inhibit intracellular germ tube formation are

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potentially able to aid in host defense against *Candida* infection. Van't Wout et al., *J. Antimicrob. Chemotherapy*, 25:803 (1990).

*Candida albicans* is grown overnight in yeast  
5 nitrogen base (Difco Laboratories, Detroit, MI)  
supplemented with 0.15% asparagine and 2% dextrose. Cells  
are pelleted and washed twice with 1x PBS.

For analysis of acute effects, the final pellet  
is resuspended in DMEM plus 2% glucose at  $5 \times 10^5$  cells/ml.  
10 200  $\mu$ l of this *Candida* suspension is added to wells in 96-  
well microtiter plates and oligonucleotides are added to  
desired concentrations. Plates are placed at 37°C under 5%  
CO<sub>2</sub> and incubated for one hour. At the end of incubation,  
glutaraldehyde is added to 0.5% and plates are chilled to  
15 4°C. Cells are examined microscopically and the percent of  
total cells with germ tubes formed is determined after  
counting three separate fields.

For analysis of effects of long-term exposure,  
cells are resuspended in YNB with 0.15% asparagine and 2%  
20 dextrose, and oligonucleotides are added. Plates are  
incubated for four hours at room temperature, after which  
cells are pelleted and washed in PBS. The final pellet is  
resuspended in DMEM plus 2% glucose and supplemented with  
fresh oligonucleotide. Cells are then incubated at 37°C  
25 under 5% CO<sub>2</sub> and the germ tube assay performed as for  
analysis of short-term effects above.

The oligonucleotide analogs tested in germ tube  
assay for inhibition of *Candida albicans* are shown in Table  
2:

TABLE 2

SEQ ID NO:	ISIS #	5'.....SEQUENCE.....3'	TARGET	TYPE
1	2214	TGT TGT CGA TAA TAT TAC CA	Chitin synthetase AUG	P=O
"	2216	" " " "	" "	P=S
2	2215	CAA TTT CTC TCA TAG TTC TA	$\beta$ -Tubulin AUG	P=O
"	2217	" " " "	" "	P=S
3	2754	TCA CTG GAT GGA GCC ATT TTC	Ribosomal L25 AUG	P=O
4	2839	CAC TGG ATG CAC CCA TTT TGT	Ribosomal L25 AUG	P=O
"	2845	" " " "	" "	P=S
5	2933	CTC ATA GTT CTA TAA TGT TGA	$\beta$ -Tubulin AUG	P=S
6	2938	TGT TGT GCA TAA TAT TAC CA	Chitin synthetase AUG	P=S
7	3156	TTT ACC CAT GAT TGA TTA TAT	TEF1 and TEF2 AUG	P=O
"	3122	" " " "	" "	P=S
8	3121	TCA CTG GAT GGA GCC ATT TTG	Ribosomal L25 AUG	P=O
9	3152	TGA CAT GAT CAA TGG ATG ACA	ATPase subunit b AUG	P=O
"	3125	" " " "	" "	P=S
10	3150	GTG CAT AAT ATT ACC ATC AAT	Chitin synthetase AUG	P=S
11	3151	AGC CAT ATT GAG TTA TGA TCT	Cytochrome P450 L1A1 AUG	P=S
12	1049	GCC GAG GTC CAT GTC GTA CGC	Control- HSV UL13	P=O
"	1082	" " " "	" "	P=S

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Figures 2 A and B show the results of a time course analysis comparing the effects of antisense oligonucleotides (phosphodiester and phosphorothioate analog) specifically hybridizable with the chitin synthetase gene of *Candida albicans* with several controls. ISIS 2214 (SEQ ID NO: 1) is the phosphodiester oligonucleotide targeted to the AUG region of chitin synthetase. ISIS 2216 (SEQ ID NO: 1) is the phosphorothioate analog of ISIS 2214. ISIS 1049 and ISIS 1082 (SEQ ID NO: 12) are the phosphorodiester and phosphorothioate analog, respectively, of a control sequence hybridizable with a translation initiation codon of the mRNA product of the herpes simplex virus UL13 gene. "Control" indicates untreated cells. Results of germ tube assays at two doses of oligonucleotides, 0.5 and 1.0  $\mu$ M, are shown in Figure 2(A) and Figure 2(B), respectively. At both doses, ISIS 2216, the phosphorothioate oligonucleotide analog hybridizable with mRNA encoding *Candida* chitin synthetase, showed a greater inhibition of *Candida* germ tube formation relative to the other compounds.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

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5

Ecker, David J.

(ii) TITLE OF INVENTION: Inhibition of Candida

(iii) NUMBER OF SEQUENCES: 12

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(E) COUNTRY: USA

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(F) ZIP: 19103

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: DISKETTE, 3.5 INCH, 1.44 Mb  
STORAGE

20

(B) COMPUTER: IBM PS/2

(C) OPERATING SYSTEM: PC-DOS

(D) SOFTWARE: WORDPERFECT 5.0

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: n/a

(B) FILING DATE: herewith

25

(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Licata, Jane M.

(B) REGISTRATION NUMBER: 32,257

(C) REFERENCE/DOCKET NUMBER: ISIS-0432

30

(ix) TELECOMMUNICATION INFORMATION:

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(B) TELEFAX: (215) 568-3439

## (2) INFORMATION FOR SEQ ID NO:1:

35

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

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(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oth r nucl ic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TGTTGTCGAT AATATTACCA

20

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CAATTTCTCT CATAGTTCTA

20

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TCACTGGATG GAGCCATTTT C

21

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucl ic acid

(C) STRANDEDNESS: singl

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CACTGGATGC ACCCATTTTG T

21

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other nucleic acid

15 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTCATAGTTC TATAATGTTG A

21

20 (2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

25 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

30 TGTTGTGCAT AATATTACCA

20

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

35 (D) TOPOLOGY: lin ar

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- (ii) MOLECULE TYPE: Other nucleic acid
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: YES
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

5

TTTACCCATG ATTGATTATA T

21

## (2) INFORMATION FOR SEQ ID NO:8:

## (i) SEQUENCE CHARACTERISTICS:

10

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: Other nucleic acid
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: YES
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

15

TCACTGGATG GAGCCATTTT G

21

## (2) INFORMATION FOR SEQ ID NO:9:

20

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

25

- (ii) MOLECULE TYPE: Other nucleic acid
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: YES
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TGACATGATC AATGGATGAC A

21

30

## (2) INFORMATION FOR SEQ ID NO:10:

## (i) SEQUENCE CHARACTERISTICS:

35

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: Other nucleic acid

- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: YES
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GTGCATAATA TTACCATCAA T

- (2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: Other nucleic acid
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: YES
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AGCCATATTG AGTTATGATC T

- (2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: Other nucleic acid
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: YES
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GCCGAGGTCC ATGTCGTACG C

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## CLAIMS

What is claim d is:

1. An oligonucleotide or oligonucleotide analog specifically hybridizable with at least a portion of mRNA which encodes  $\beta$ -tubulin, actin, chitin synthetase, aspartate protease, translation elongation factor 1, translation elongation factor 2, ribosomal L25 protein, ATPase b subunit, or cytochrome P450 lanosterol 14 $\alpha$ -demethylase protein of a *Candida*.
2. The oligonucleotide or oligonucleotide analog of claim 1 specifically hybridizable with at least a portion of a transcription initiation site, a translation initiation site, an intron/exon junction, or the 5' cap region of the mRNA.
3. The oligonucleotide or oligonucleotide analog of claim 1 in a pharmaceutically acceptable carrier.
4. The oligonucleotide or oligonucleotide analog of claim 1 having from 5 to about 50 nucleic acid base units.
5. The oligonucleotide or oligonucleotide analog of claim 1 having from 8 to about 25 nucleic acid base units.
6. The oligonucleotide or oligonucleotide analog of claim 1 having from 12 to about 25 nucleic acid base units.
7. The oligonucleotide or oligonucleotide analog of claim 1 wherein at least some of the linking groups between nucleotide units of the oligonucleotide comprise sulfur-containing species.
8. The oligonucleotide or oligonucleotide analog of claim 1 wherein at least some of the linking groups between nucleotide units of the oligonucleotide comprise phosphorothioate moieties.

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9. The oligonucleotide or oligonucleotide analog of claim 1 wherein the mRNA encodes the  $\beta$ -tubulin protein.

10. An oligonucleotide or oligonucleotide analog specifically hybridizable with an RNA of *Candida* and comprising at least a portion of one of the sequences

CAA TTT CTC TCA TAG TTC TA,  
CGG AAC ATA CAA TTT CTC TC,  
CAA AAG CAG TTA GTA TAT TT,  
10 AAA AAT TGT TAG TAA AAT CA,  
CTA AAA AAA AGG GCA AAA GC,  
TTC CCA AAA GGC AGC ACC CT,  
ATG ATA ACT GCA TGA TGT TG,  
GGA AGG ATT CCC GTG TGC GG,  
15 AAC AAT ACC TAA ACC TTG GA,  
ACC ACC GTC CAT TTT GAA TG,  
TTA AAA CAT ACA CCG TCC A,  
CTA TAA AAA TGG GTT GTA AT,  
TGT TGT CGA TAA TAT TAC CA,  
20 GTG TAT GTC ATG TTG GTA AA, or  
TTT AGC TCT AAC ATC ACC AC.

11. The oligonucleotide or oligonucleotide analog of claim 10 in a pharmaceutically acceptable carrier.

12. The oligonucleotide or oligonucleotide analog of claim 10 wherein at least some of the linking groups between nucleotide units of the oligonucleotide comprise sulfur-containing species.

13. The oligonucleotide or oligonucleotide analog of claim 10 wherein at least some of the linking groups between nucleotide units of the oligonucleotide comprise phosphorothioate moieties.

14. A method for treating a *Candida* infection comprising contacting an animal suspected of having a *Candida* infection with an oligonucleotide or oligonucleotide analog specifically hybridizable with at least a portion of mRNA which encodes  $\beta$ -tubulin, actin, chitin



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synthetase, aspartate protease, translation elongation factor 1, translation elongation factor 2, ribosomal L25 protein, ATPase b subunit, or cytochrome P450 lanosterol 14 $\alpha$ -demethylase protein of *Candida*.

5           15. The method of claim 14 wherein the oligonucleotide or oligonucleotide analog is specifically hybridizable with at least a portion of a transcription initiation site, a translation initiation site, an intron/exon junction, or the 5' cap region of the mRNA.

10           16. The method of claim 14 wherein the oligonucleotide or oligonucleotide analog is in a pharmaceutically acceptable carrier.

          17. The method of claim 14 wherein the oligonucleotide or oligonucleotide analog has from 5 to  
15 about 50 nucleic acid base units.

          18. The method of claim 14 wherein the oligonucleotide or oligonucleotide analog has from 8 to about 25 nucleic acid base units.

          19. The method of claim 14 wherein the  
20 oligonucleotide or oligonucleotide analog has from 12 to about 25 nucleic acid base units.

          20. The method of claim 14 wherein at least some of the linking groups between nucleotide units of the oligonucleotide or oligonucleotide analog comprise sulfur-  
25 containing species.

          21. The method of claim 14 wherein at least some of the linking groups between nucleotide units of the oligonucleotide or oligonucleotide analog comprise phosphorothioate moieties.

30           22. The method of claim 14 wherein the mRNA encodes the  $\beta$ -tubulin protein.

          23. The method of claim 14 wherein the infection is of *Candida albicans*, *Candida tropicalis*, *Candida krusei*, *Torulopsis glabrata* or *Candida parapsilosis*.

35           24. A method for modulating the activity of *Candida* comprising contacting an animal suspected of having a *Candida* infection with an oligonucleotide or oligonucleo-

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tide analog comprising at least a portion of one of the sequences:

5 CAA TTT CTC TCA TAG TTC TA,  
 CGG AAC ATA CAA TTT CTC TC,  
 CAA AAG CAG TTA GTA TAT TT,  
 AAA AAT TGT TAG TAA AAT CA,  
 CTA AAA AAA AGG GCA AAA GC,  
 TTC CCA AAA GGC AGC ACC CT,  
 ATG ATA ACT GCA TGA TGT TG,  
 10 GGA AGG ATT CCC GTG TGC GG,  
 AAC AAT ACC TAA ACC TTG GA,  
 ACC ACC GTC CAT TTT GAA TG,  
 TTA AAA CAT ACA CCG TCC A,  
 CTA TAA AAA TGG GTT GTA AT,  
 15 TGT TGT CGA TAA TAT TAC CA,  
 GTG TAT GTC ATG TTG GTA AA, or  
 TTT AGC TCT AAC ATC ACC AC.

25. An oligonucleotide or oligonucleotide analog specifically hybridizable with an RNA of *Candida* and  
 20 comprising at least a portion of one of the sequences identified in Table 2.

26. The oligonucleotide or oligonucleotide analog of claim 25 in a pharmaceutically acceptable carrier.

25 27. The oligonucleotide or oligonucleotide analog of claim 25 wherein at least some of the linking groups between nucleotide units of the oligonucleotide comprise sulfur-containing species.

28. The oligonucleotide or oligonucleotide  
 30 analog of claim 25 wherein at least some of the linking groups between nucleotide units of the oligonucleotide comprise phosphorothioate moieties.

29. A method for modulating the activity of  
 35 *Candida* comprising contacting an animal suspected of having a *Candida* infection with a therapeutically effective amount of an oligonucleotide or oligonucleotide analog comprising

- 25 -

at least a portion of one of the sequences identified in Table 2.

30. The method of claim 29 wherein at least some of the linking groups between nucleotide units of the  
5 oligonucleotide or oligonucleotide analog comprise sulfur-containing species.

31. The method of claim 29 wherein at least some of the linking groups between nucleotide units of the oligonucleotide or oligonucleotide analog comprise  
10 phosphorothioate moieties.

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FIG. 1

1 TTATATCAAA TAGATTTAGA TTTTTTTATT TTAAAGAATT TTTTAATCAA  
51 GAAATCAATA TCAACATTAT AGAACTATGA GAGAAATTGT ATGTTCCGTT  
101 TATTCCCTTC CTTACACCAA ATCATTGGAA TCCTTATGTG TTTTGTGTGT  
151 GTTGTCTGAA AATTTTTGGT TCTGTTTTAC GCGCCTTTTT CGACTAAATT  
201 GATTCAAATA GGATTCCCTA AATGATTAAAT GATTGTGTGC AATCAATCAA  
251 TGTTTTATTA AGTTTTATCA AATATACTAA CTGCTTTTGC CCTTTTTTTT  
301 AGATTCATTT ATCAACTGGT CAATGTGGTA ATCAAATTGT ATGTATAAAC  
351 ACTGAAGAAA AAAAAATTCT ATCATTGTTA TGTTGTGATC TTTGATCTTT  
401 AGTTGTCGGG TTAACACCTG CCAATTGGAT CAATACATCA ATCAATTAAT  
451 TCTAATCTTG AAAAAAAAAAT TGATTTTACT AACAAATTTT TCTTTTATTT  
501 AGGGTGCTGC CTTTTGGGAA ACTATTGTG GAGAACATGG ATTAGATAAC  
551 AATGGAAGTT ATGTTGAAA TAATGAACTT CAAAAATCCA AATTAGACGT  
601 TTATTTCAAC GAAGCTACTT CTGGGAAATA CGTTCCTCGT GCCGTTTTAG  
651 TCGATTTGGA ACCAGGTACT ATTGATAATG TGAAAACTTC ACAAATTGGT  
701 AACTTGTTTA GACCAGATAA CTTTATTTTC GGTCAAAGTT CTGCCGGCAA  
751 TGTTTGGGCT AAAGGTCATT AACTGAAGG TGCTGAATTA GTTGATTCTG  
801 TTTTAGATGT TGTTAGAAGA GAAGCTGAAG GCTGTGATTC TTTACAAGGT  
851 TTCAAATCA CCCATTCTTT GGGTGCTGGT ACTGGTTCTG GTATGGGTAC  
901 TTTGTTGATT TCTAAAATTA GAGAAGAATT CCCTGATACA ATGATGGCCA  
951 CTTTTTCTGT TGTCCCATCA CAAAAGTTT CCGATACCGT TATTGAACCA  
1001 TATAACGCTA CTTTATCAGT CCATCAATTG GTTGAAAACT CTGATGAAAC  
1051 TTTCTGTATT GATAATGAAG CCTTGTACAA TATTTGTCAA AACACTTTGA  
1101 AATTACCACA ACCATCTTAT GCTGAATTGA ACAATTTGGT TTCTTCTGTC  
1151 ATGTCTGGTG TTACTACTTC TTTACGTTAT CCAGGTCAAT TGAATTCGGA  
1201 TTTAAGAAAA TTGGCAGTCA ATTTGGTTCC ATTCCCAAGA TTACATTTCT

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1251 TTATGGTTGG TTATGCTCCA TTGACTTCTA TGGGTTCTAA ATCTTTCAGA  
1301 TCAGTCACCG TCCCAGAATT GACTCAACAA ATGTTTGATG CCAAAAATAT  
1351 GATGGCTGCT TCTGATCCAA GAAATGGTCG TTATTAACT GTTGCTGCCT  
1401 TTTTCAGAGG TAAAGTATCT GTTAAAGAAG TTGACGATGA AATGCACAAA  
1451 ATCCAAACCA GAAACTCATC TTATTTTGTT GATTGGATTC CAAATAATGT  
1501 TCAAACCTGCT GTTTGTTTCTG TTCCTCCAAA AGATTGATG ATGTCTGCTA  
1551 CTTTATTGG AACTCTACT TCCATTCAAG AATTATTTAA AAGAGTTGGT  
1601 GATCAATTCA GTGCTATGTT CAGAAGAAAA GCTTCTTGC ATTGGTATAC  
1651 TTCTGAAGGT ATGGATGAAA TGAATTTAC TGAAGCTGAA TCTAATATGA  
1701 ATGATTTGGT TAGTGAATAC CAACAATACC AAGAAGCTAG TATTGATGAA  
1751 GAAGAATTAG AATATGCCGA TGAAATCCCA TTAGAAGATG CCGCCATGGA  
1801 ATAAAAGCTG ATAAATGCTA CAATATTAAT TAATTATAAT TTTTTTTTTT  
1851 GTTCACTTCT AATATAATTA TGGTTTTTTT TGGTTTAG

3/3

FIG. 2A

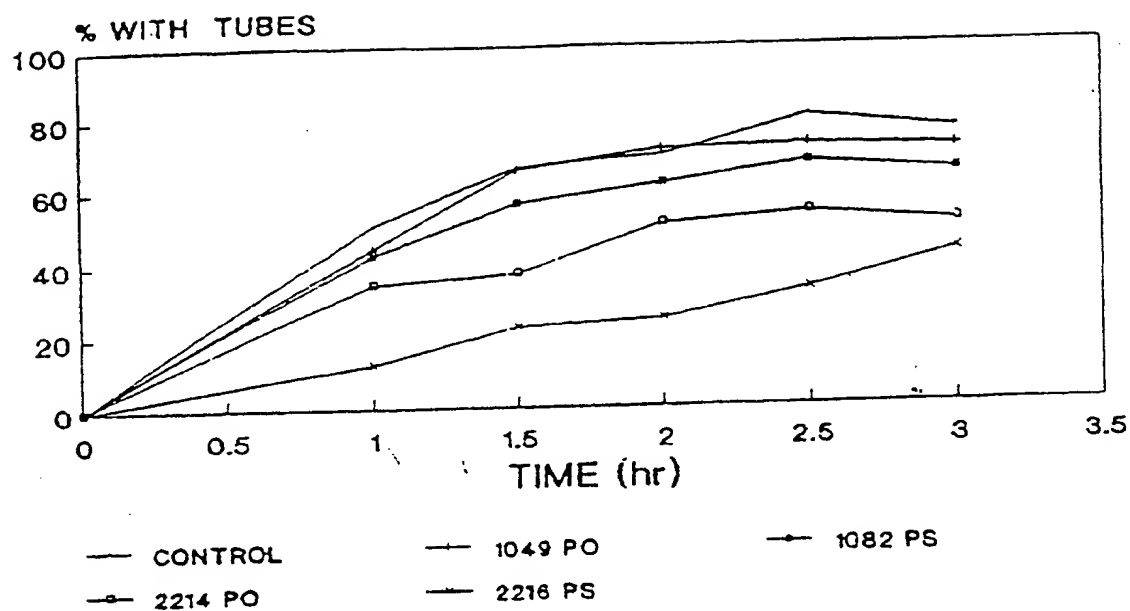
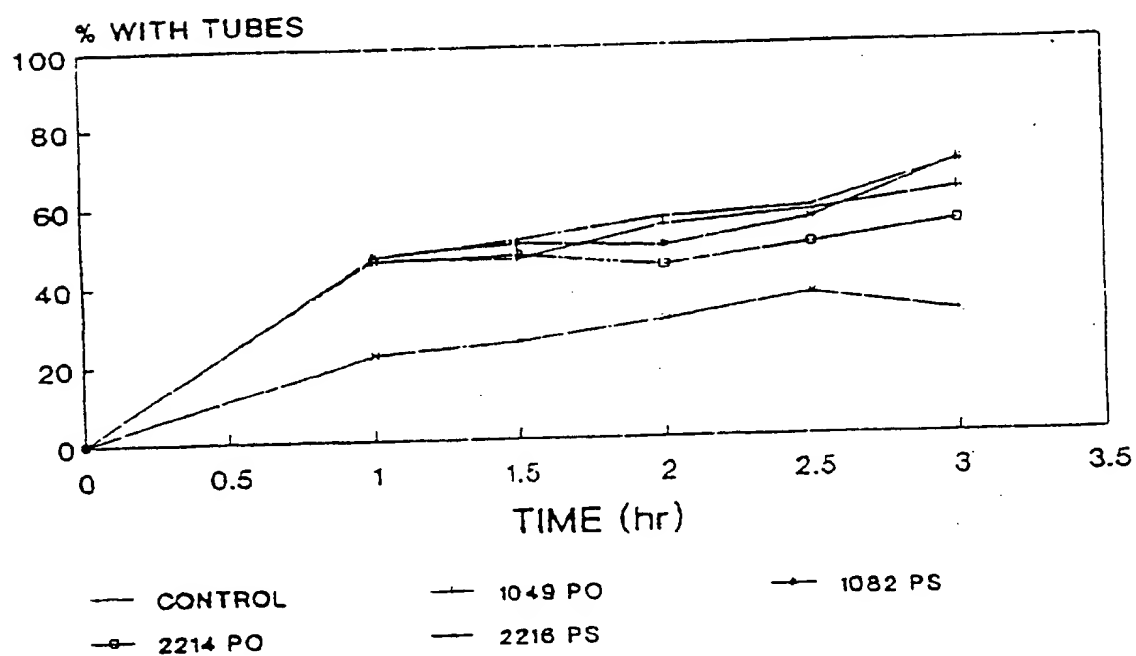


FIG. 2B



# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 91/05802

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>6</sup> According to International Patent Classification (IPC) or to both National Classification and IPC IPC(5): C07H 15/12; A01N 37/18; A61K 37/00 U.S.C1: 536/27, 28, 29; 514/2											
<b>II. FIELDS SEARCHED</b> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Minimum Documentation Searched <sup>7</sup></div> <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 30%; border: 1px solid black; text-align: left;">Classification System</th> <th style="border: 1px solid black; text-align: left;">Classification Symbols</th> </tr> <tr> <td style="border: 1px solid black; padding: 5px;">U.S.CL:</td> <td style="border: 1px solid black; padding: 5px;">536/27, 28, 29; 514/2</td> </tr> </table> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>8</sup></div> <p>Databases: STN (CA) Automated Patent System (File US Pat 1971-1991)</p>			Classification System	Classification Symbols	U.S.CL:	536/27, 28, 29; 514/2					
Classification System	Classification Symbols										
U.S.CL:	536/27, 28, 29; 514/2										
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT <sup>9</sup></b> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 10%; text-align: left;">Category <sup>*</sup></th> <th style="width: 70%; text-align: left;">Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup></th> <th style="width: 20%; text-align: left;">Relevant to Claim No. <sup>13</sup></th> </tr> </thead> <tbody> <tr> <td style="vertical-align: top; padding: 5px;">Y</td> <td style="vertical-align: top; padding: 5px;">Nuclear Acids Research, Vol. 17, number 22, issued 1989. Losberger et al.. "Sequence of the Candida albicans gene encoding actin", page 9488. see entire document.</td> <td style="vertical-align: top; padding: 5px;">1-31</td> </tr> <tr> <td style="vertical-align: top; padding: 5px;">Y</td> <td style="vertical-align: top; padding: 5px;">Gene, vol. 63, issued 1988. Smith et al.. "Isolation and characterization of a B-tubulin gene from Candida albicans", pages 53-63. see especially page 57.</td> <td style="vertical-align: top; padding: 5px;">1-31</td> </tr> </tbody> </table>			Category <sup>*</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>	Y	Nuclear Acids Research, Vol. 17, number 22, issued 1989. Losberger et al.. "Sequence of the Candida albicans gene encoding actin", page 9488. see entire document.	1-31	Y	Gene, vol. 63, issued 1988. Smith et al.. "Isolation and characterization of a B-tubulin gene from Candida albicans", pages 53-63. see especially page 57.	1-31
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<div style="display: flex; justify-content: space-between;"> <div style="width: 48%;"> <p><sup>*</sup> Special categories of cited documents: <sup>10</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 48%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance: the claim invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&amp;" document member of the same patent family</p> </div> </div>											
<b>IV. CERTIFICATION</b> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; border-bottom: 1px solid black; padding: 5px;">           Date of the Actual Completion of the International Search  <div style="text-align: center;">01 November 1991</div> </td> <td style="width: 50%; border-bottom: 1px solid black; padding: 5px;">           Date of Mailing of this International Search Report  <div style="text-align: center; font-size: 1.2em;">18 DEC 1991</div> </td> </tr> <tr> <td style="border-bottom: 1px solid black; padding: 5px;">           International Searching Authority  <div style="text-align: center;">ISA/US</div> </td> <td style="border-bottom: 1px solid black; padding: 5px;">           Signature of Authorized Officer  <div style="text-align: center;">Gian Wang <i>Gian Wang</i></div> </td> </tr> </table>			Date of the Actual Completion of the International Search <div style="text-align: center;">01 November 1991</div>	Date of Mailing of this International Search Report <div style="text-align: center; font-size: 1.2em;">18 DEC 1991</div>	International Searching Authority <div style="text-align: center;">ISA/US</div>	Signature of Authorized Officer <div style="text-align: center;">Gian Wang <i>Gian Wang</i></div>					
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International Searching Authority <div style="text-align: center;">ISA/US</div>	Signature of Authorized Officer <div style="text-align: center;">Gian Wang <i>Gian Wang</i></div>										

## FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	Gene, vol. 72 issued 1988. Inouye, "Antisense RNA: its functions and applications in gene regulation-a review", pages 25-34, see entire document.	1-31
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V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE<sup>1</sup>

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers \_\_\_\_\_, because they relate to subject matter<sup>12</sup> not required to be searched by this Authority, namely:
  
2. ☐ Claim numbers \_\_\_\_\_, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out<sup>13</sup>, specifically:
  
3. ☐ Claim numbers \_\_\_\_\_, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING<sup>2</sup>

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

## Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.  
☐ No protest accompanied the payment of additional search fees.